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14. ABSTRACT
Current antidotes for cyanide poisoning must be administered by intravenous injection, which would not be practical for treating mass casualties as could occur in a major industrial accident or a terrorist attack. Thus, a need exists for alternative modes of administering cyanide antidotes, and we are comparing three different administration modes: intramuscular injection, preferably via an autoinjector, intraosseous injection, and inhalational delivery. We found that all three modes can rescue animals from exposure to lethal cyanide doses. As part of these studies, we tested three new cyanide antidotes that under development: cobinamide, dimethyltrisulfide, and sulfanegen.

15. SUBJECT TERMS
Cyanide Poisoning; Intramuscular Injection; Intraosseous Injection; Inhalational Delivery

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INTRODUCTION

Cyanide is a rapidly acting poison, and, thus, antidotes must be administered quickly; clearly, the fastest way to deliver a drug to the systemic circulation is via intravenous injection. However, even under the best of circumstances, starting an intravenous line takes several minutes, and even more time will likely be required in cyanide-poisoned victims, since they may be hypotensive with collapsed peripheral veins. Thus, intravenous antidote administration would not be practical in the setting of mass casualties from cyanide exposure. Of other possible routes of drug administration, three stand out as potentially very useful to treat a large number of cyanide-poisoned people: intramuscular injection, intraosseous injection, and inhalational delivery. We are comparing each of these three drug delivery modes to intravenous injection in three different species—mice, rabbits, and pigs. Each delivery mode has advantages and disadvantages. Intramuscular injection using an autoinjector is very quick, can be performed through clothing, can be done via self-administration, and has well developed technology. However, a limited volume can be injected, and muscle blood flow is relatively low under resting conditions and can be severely compromised during hypotension as occurs in cyanide poisoning. Intraosseous injection provides access to the systemic circulation as rapidly as intravenous injection, does not require finding a vein, and can be accomplished in a clothed hypotensive person. Disadvantages of intraosseous injection are that it is technically more difficult and time-consuming than intramuscular injection, cannot be conducted in a self-administration mode, and absorption can be potentially reduced by peripheral vasoconstriction. Inhalational delivery has advantages of self-administration, and very rapid absorption not compromised by peripheral vasoconstriction. Disadvantages of inhalational delivery are that the subject needs to be breathing, significant amounts of drug can be loss in the upper airways, and current inhalers deliver only small quantities of drug.

BODY

Intramuscular Injection

We tested cobinamide, dimethyltrisulfide, and sulfanegen administered by intramuscular injection in lethal mouse, rabbit, and pig models of cyanide poisoning. We found that all three drugs rescue the animals—even when the animals have been apneic for one minute—when the drugs are administered by intramuscular injection. Each drug requires a specific formulation for effective absorption after intramuscular injection. In Figure 1 (next page), we show the results for cobinamide (formulated with four moles of sodium nitrite) in an inhalational mouse model. As can be seen, nitrocobinamide fully rescued animals from a lethal exposure of cyanide gas. Sodium nitrite, at a dose corresponding to the amount of nitrite in the nitrocobinamide, had minimal effect.

Ideally, it would be best to use an autoinjector for intramuscular injection of the three cyanide antidotes, since it would be quicker and easier than using a conventional syringe and needle. We had entered into negotiations with Meridian Medical Technologies (MMT), a major provider of autoinjectors. However, MMT developed technical problems with one of its products, and has devoted all its efforts to resolving those problems. Hence, MMT is no longer a viable source of autoinjectors. We recently learned that Scandinavian Health Limited (SHL) Group markets several autoinjectors that would be appropriate for injecting a cyanide antidote. We are now discussing with them the possibility of using one of their autoinjectors in our drug development projects.

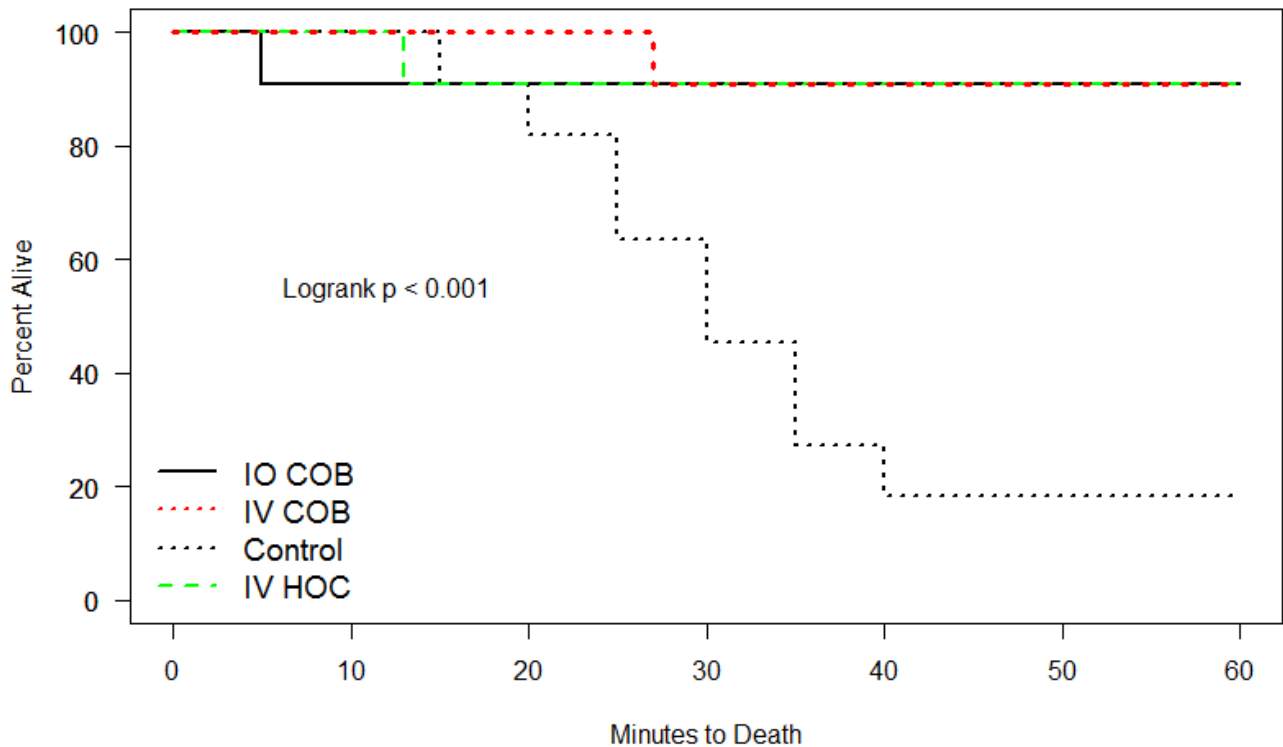**Figure. 1. Intramuscular Injection of Nitrocobinamide in Mice.** C57/Bl mice (6 animals/condition) were exposed to 587 ppm of cyanide gas for a total of 40 min in a sealed Plexiglas chamber. After 15 min of exposure, they were removed from the chamber, and injected (Inj) intramuscularly with either 1.5 μmol nitrocobinamide (red circles), 6.0 μmol sodium nitrite (green squares), or saline (purple triangles). They were then placed back in the chamber for 25 min. The 6 μmol of sodium nitrite corresponds to the amount of nitrite in the 1.5 μmol of nitrocobinamide.
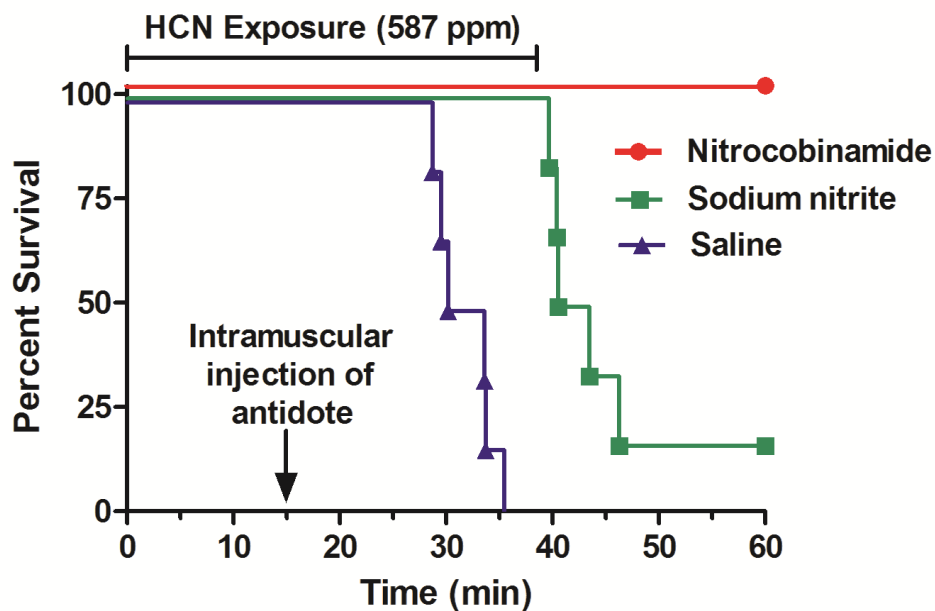
Intraosseous Injection

We have administered cobinamide via intraosseous injection to both rabbits (Drs. Brenner and Mahon) and pigs (Dr. Vikhyat Bebarta at University of Texas, San Antonio). We found that this administration mode rescued animals from lethal cyanide doses as effectively as when the antidote was given by intravenous injection. Results in pigs are shown in Figure 2 (next page). As part of these experiments, we compared cobinamide to hydroxocobalamin and found that cobinamide was five times more potent than hydroxocobalamin, i.e., 12.5 mg/kg of cobinamide was equivalent to 65 mg/kg hydroxocobalamin.

60 Minute Survival by Cyanide Treatment Groups

Logrank p < 0.001

Legend:
- IO COB
- IV COB
- Control
- IV HOC

Y-axis: Percent Alive
X-axis: Minutes to Death

Figure. 2. Intraosseous Injection of Cobinamide Rescues Pigs from Cyanide Poisoning. Cyanide was given by continuous intravenous injection to 50 kg Yorkshire pigs until one minute beyond the onset of apnea, i.e., apnea plus one minute. The animals then received saline by intraosseous injection (Control), 12.5 mg/kg cobinamide (COB) by intraosseous or intravenous injection, or 65 mg/kg hydroxocobalamin (HOC) by intravenous injection. The intraosseous injection was into the tibial plateau using a standard intraosseous injection device. Animals were randomly assigned to each group. A sample size of 11 animals per group was selected based on obtaining a power of 80%, an alpha of 0.05, and an effect size of ≥ 0.25 difference (one standard deviation) in mean time to spontaneous breathing. Time to spontaneous breathing and survival were compared using rank methods. Baseline weights (53, 51, 51 kg), time to apnea (10:54, 10:07, 9:49 min), and cyanide dose at apnea (1.8, 1.7, 1.7 mg/kg) were similar. At the time of antidote injection, mean blood cyanide (1.7, 1.7, 1.84 mcg/ml) and lactate concentrations (3.5, 3.5, 3.1 mmol/L), and reduction in mean arterial pressure MAP from baseline (29%, 28%, 36% decrease) were similar. Two of 11 animals in the saline control group survived (18% survival) compared to 10 of 11 surviving in both the intravenous and intraosseous cobinamide groups (91% survival) (p<0.001 difference between saline- and cobinamide-injected animals). Survival in the intravenous hydroxocobalamin group was also 91%. Time to spontaneous breathing after antidote was similar (intravenous 1:48 min, and intraosseous 1:47 min). Blood cyanide concentrations became undetectable after cobinamide infusion in both groups. No statistically significant differences were detected between the intraosseous and intravenous cobinamide groups for cardiac output, MAP, or minute ventilation, or in lactate (1.3 vs 1.8 mmol/L) and pH (7.44 vs 7.41) at 60 min. We conclude that intraosseous cobinamide is as effective as intravenous cobinamide for treating cyanide-poisoned pigs.

Inhalational Delivery

Dr. Chen Tsai at the University of California, Irvine has further developed an ultrasonic nebulizer to deliver cyanide antidotes by inhalation (Figure 3, next page). It is a small, pocket-sized device (8.6 x 5.6 x 1.5 cm^3) that contains a nozzle, electronic driver, cell-phone battery, piezoelectric micro pump, drug reservoir, and liquid feed system. It can deliver flow rates of up to 0.2 ml/min, allowing a cyanide antidote to be given in about five minutes. We have shown that giving cobinamide by this device can successfully rescue rabbits from lethal cyanide doses. During the past year, an improved nozzle platform was designed and fabricated. The new platform is considerably smaller in size and capable of incorporating a pair of centimeter-sized nozzles that will facilitate a higher delivery rate for a single antidote or delivery of separate antidotes such as cobinamide and sulfanegen. Dr. Tsai recently had a paper accepted at the annual meeting of the Institute of Electrical and Electronics Engineers (IEEE) describing this new ultrasonic nebulizer. In Figure 3, we present data using this nebulizer, showing that it delivers cobinamide quickly to cyanide-poisoned rabbits as evidenced by almost immediate reversal in oxy- and deoxyhemoglobin concentrations.

Fig. 3. Inhalational Drug Delivery in Rabbits. New Zealand white rabbits received a continuous intravenous infusion of cyanide starting at time zero. This caused the oxyhemoglobin concentration to rise and the deoxyhemoglobin concentration to fall. At the indicated time, cobinamide was administered by inhalation through the animal's nasotracheal tube. Note the immediate reversal in the oxy- and deoxyhemoglobin concentrations; this is similar to what is observed after intravenous injection of drug.
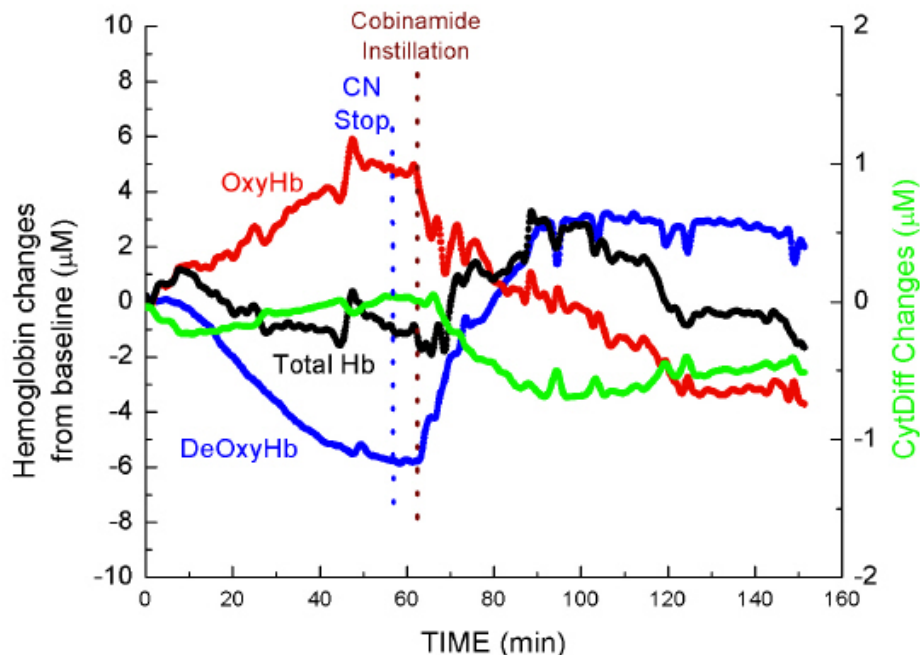
KEY RESEARCH ACCOMPLISHMENTS

Cyanide antidotes delivered by intramuscular injection, intraosseous injection, and inhalation using a high flow novel ultrasonic nebulizer effectively rescue animals from lethal cyanide poisoning. Thus, all three delivery modes are viable routes to administer cyanide antidotes.

REPORTABLE OUTCOMES

Three reportable outcomes were generated during the last year of the grant: a paper is in press in Annals of Emergency Medicine, a paper is in press in the IEEE International Ultrasonics Symposium, and a paper has been submitted.

1. Bebarta, V., Boudreau, S., Dixon, P., Boss, G. R. Intravenous cobinamide versus hydroxocobalamin for acute treatment of severe cyanide poisoning in a swine (Sus Scrofa) model. *Annals of Emergency Medicine*, in press, 2014

2. Mao, R.W., Lin, S.K., Tsai, S.C., Brenner, M., Mahon, S., Boss, G., Smaldone, G., Tsai, C.S. MEMS-Based Silicon Ultrasonic Twin-Nozzle Nebulizer for Inhalation Drug Delivery. IEEE International Ultrasonics Symposium Proceedings, 2013, in press.

3. Bebarta, V, Tanen,D. Boudreau,S.M., Castaneda,M.G. Tone, T., and Boss, G.R. Intraosseous versus intravenous cobinamide in treating acute cyanide toxicity and apnea in a swine (Sus Scrofa) model. Manuscript submitted.

CONCLUSION

We have shown that cyanide antidotes can be administered by intramuscular injection, by intraosseous injection, and by inhalational delivery. By all three modes, animals can be rescued from lethal doses of cyanide.

REFERENCES

Not applicable.

APPENDICIES

The two in press papers reported above are included.

TOXICOLOGY/ORIGINAL RESEARCH

Intravenous Cobinamide Versus Hydroxocobalamin for Acute Treatment of Severe Cyanide Poisoning in a Swine (*Sus scrofa*) Model

Lt Col Vikhyat S. Bebarta, MC, USAF*; David A. Tanen, MD; Susan Boudreau, RN, BSN; Maria Castaneda, MS;
Lee A. Zarzabal, MS; Toni Vargas, PA-C; Gerry R. Boss, MD

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Study objective: Hydroxocobalamin is a Food and Drug Administration–approved antidote for cyanide poisoning. Cobinamide is a potential antidote that contains 2 cyanide-binding sites. To our knowledge, no study has directly compared hydroxocobalamin with cobinamide in a severe, cyanide-toxic large-animal model. Our objective is to compare the time to return of spontaneous breathing in swine with acute cyanide-induced apnea treated with intravenous hydroxocobalamin, intravenous cobinamide, or saline solution (control).

Methods: Thirty-three swine (45 to 55 kg) were intubated, anesthetized, and instrumented (continuous mean arterial pressure and cardiac output monitoring). Anesthesia was adjusted to allow spontaneous breathing with FiO_2 of 21% during the experiment. Cyanide was continuously infused intravenously until apnea occurred and lasted for 1 minute (time zero). Animals were then randomly assigned to receive intravenous hydroxocobalamin (65 mg/kg), cobinamide (12.5 mg/kg), or saline solution and monitored for 60 minutes. A sample size of 11 animals per group was selected according to obtaining a power of 80%, an α of .05, and an SD of 0.17 in mean time to detect a 20% difference in time to spontaneous breathing. We assessed differences in time to death among groups, using Kaplan-Meier estimation methods, and compared serum lactate, blood pH, cardiac output, mean arterial pressure, respiratory rate, and minute ventilation time curves with repeated-measures ANOVA.

Results: Baseline weights and vital signs were similar among groups. The time to apnea and cyanide dose required to achieve apnea were similar. At time zero, mean cyanide blood and lactate concentrations and reduction in mean arterial pressure from baseline were similar. In the saline solution group, 2 of 11 animals survived compared with 10 of 11 in the hydroxocobalamin and cobinamide groups ($P<.001$ between the 2 treated groups and the saline solution group). Time to return of spontaneous breathing after antidote was similar between hydroxocobalamin and cobinamide (1 minute 48 seconds versus 1 minute 49 seconds, respectively). Blood cyanide concentrations became undetectable at the end of the study in both antidote-treated groups, and no statistically significant differences were detected between the 2 groups for mean arterial pressure, cardiac output, respiratory rate, lactate, or pH.

Conclusion: Both hydroxocobalamin and cobinamide rescued severely cyanide-poisoned swine from apnea in the absence of assisted ventilation. The dose of cobinamide was one fifth that of hydroxocobalamin. [Ann Emerg Med. 2014;■:1-8.]

Please see page XX for the Editor's Capsule Summary of this article.

INTRODUCTION

Background

Hydroxocobalamin is a Food and Drug Administration–approved antidote for treating acute cyanide poisoning.[1-3] However, because of its poor water solubility, it must be administered in a relatively large volume intravenously. In the out-of-hospital setting of cyanide gas release, such as an industrial accident, terrorist attack, or commercial or residential fire, intravenous access can be difficult and particularly problematic for providers wearing hazardous material protective gear; thus, nonintravenous antidotes are urgently needed.[4-7] To address this concern, federal agencies, including the National Institutes of Health and the US Army Medical Research Institute of Chemical Defense, are actively seeking nonintravenous, potent new antidotes.[4,5] Cobinamide is a water-soluble analog of hydroxocobalamin that has a much higher affinity for cyanide than hydroxocobalamin and binds 2 moles of cyanide per mole compared with 1 mole of cyanide for each mole of hydroxocobalamin. Cobinamide has been shown to be 3 to 10 times more potent than hydroxocobalamin[6] in both mice and rabbit models of acute cyanide intoxication,[7,8] but to our knowledge it has not been studied in a critically ill large-animal model of cyanide poisoning.

Editor's Capsule Summary

What is already known on this topic

An intramuscularly administered antidote for cyanide toxicity would be desirable, particularly in the out-of-hospital environment. Cobinamide is a candidate agent, but its intravenous efficacy has not yet been evaluated.

What question this study addressed

This nonblinded 33-pig study compared the efficacy of intravenous cobinamide, hydroxocobalamin, and placebo in a nonlethal model of cyanide poisoning.

What this study adds to our knowledge

The primary endpoint, time to return of spontaneous respirations after intravenous administration, was similar for both antidotes.

How this is relevant to clinical practice

If findings are confirmed with intramuscular administration, cobinamide may be an effective out-of-hospital antidote, especially during mass toxicologic events.

Goals of This Investigation

Our goal was to compare the time to return of spontaneous breathing among 3 groups of swine after 1 minute of acute cyanide-induced apnea. This model has been previously validated as a model of acute cyanide toxicity and was developed to have a potential low survival rate in untreated animals.[3,9,10] If intravenous cobinamide is as efficacious as hydroxocobalamin in treating cyanide poisoning in a large animal, future studies could test the efficacy of cobinamide administered by the intramuscular route.

MATERIALS AND METHODS

Study Design and Setting

This investigation was a nonblinded randomized study approved by the Wilford Hall Clinical Research Division Institutional Animal Care and Use Committee. All animal experiments complied with the regulations and guidelines of the Animal Welfare Act, the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and the American Association for Accreditation of Laboratory Animal Care. Animals were housed and the study was conducted in the Animal Care Facility.

Before the beginning of the experiment, animals were randomly assigned, using a commonly used online medical research randomization plan generator (http://www. randomization.com) to one of the 3 following intravenous

interventions: (1) hydroxocobalamin, the positive control and standard antidote; (2) cobinamide, the experimental antidote; or (3) saline solution, the negative control.

Yorkshire swine (*Sus scrofa*) (n=33, weighing 45 to 55 kg, female) were premedicated with intramuscular ketamine 10 mg/kg. General anesthesia was induced with isoflurane by nose cone. After intubation, the animals were mechanically ventilated with a volume-limited, time-cycled ventilator (Fabius GS anesthesia machine; Drager-Siemens, New York, NY) and maintained with inhaled isoflurane (1% to 3%) and oxygen (FiO_2 of 0.4 to 0.45). The tidal volume was initially 8 to 10 mL/kg and respiratory rate was 12 breaths/min. The minute ventilation was adjusted to maintain an end tidal CO_2 of 38 to 42 mm Hg as measured by inline capnography. Lead II of the surface ECG was monitored continuously. Animal body temperature was maintained at 37.5°C (99.5°F) to 39.0°C (102.2°F). Baseline biochemical variables (arterial blood gas, hemoglobin, and electrolytes) were measured.

Interventions

Invasive hemodynamic variables were measured with an 8-French Swan-Ganz CCOmbo pulmonary artery catheter (model 746HF8) and the Edwards Vigilance II monitor (Edwards Lifesciences, Irvine, CA). Measurements included continuous cardiac output, systemic vascular resistance, mixed venous oxygen saturation, central venous pressure, pulmonary artery pressure, and core temperature. Catheter ports were flushed with saline solution, and the catheter was placed by cutdown in the right external jugular. Aortic pressure was measured continuously through the femoral artery. An 8.5-French introducer (Arrow, Reading, PA) was placed in the carotid artery for laboratory sampling and another was placed in the femoral vein for medication administration. The animals received a warmed saline solution intravenous bolus (15 mL/kg) during procedure setup. Heparin (100 U/kg) was administered intravenously after catheters were inserted. The Fabius GS anesthesia data collection software embedded in the ventilator's computer was used for data acquisition at 1-minute intervals.

Baseline biochemical measurements included oxygen saturation, $PaCO_2$, PaO_2, and pH (ABL 800 Flex blood gas analyzer; Radiometer America, Westlake, OH), hemoglobin (OSM3 Hemoximeter; Radiometer, Westlake, OH), and electrolytes (Piccolo Chemistry Analyzer; Abaxis, Union City, CA). Ventilation and oxygenation variables were also collected and included tidal volume, respiratory rate, minute volume, and pulse oximetry.

After a 10-minute acclimation period, isoflurane was reduced to 1% to 1.5%, the FIO_2 was adjusted to room air (0.21), and the mechanical ventilator was turned off. Thus, the animals breathed spontaneously for the remainder of the experiment. Once the animals had sustained spontaneous respirations for 5 minutes, a 0.4% potassium cyanide solution (potassium cyanide; Sigma Aldrich, St. Louis, MO; normal saline solution) was infused continuously until apnea occurred and was confirmed by capnography for 20 seconds. At this point (apnea),

the cyanide infusion was stopped. After capnographic confirmation of 1 additional minute of apnea (time zero), animals were administered the antidote or saline solution intravenously as a bolus injection. The cobinamide was infused in less than 1 minute and the hydroxocobalamin was infused during 2 to 3 minutes to administer the larger volume. Animals received equal volumes of 90 mL during the infusion with 10 mL of saline solution given before and 10 mL infused after each drug administration. Cobinamide was infused in 4 to 8 mL, with an additional 80 to 85 mL of saline solution, hydroxocobalamin was infused in 90 mL, and 90 mL of saline solution was infused for control animals. The dose and infusion duration for cobinamide and hydroxocobalamin were based on previous published animal models and preliminary experiments in our laboratory.[3,8-10]

The animals were monitored for 60 minutes after treatment. Death was defined as a mean arterial pressure less than 20 mm Hg for 5 minutes. Animals that died were observed for an additional 20 minutes or until the end of the experiment to evaluate for a possible delayed therapeutic effect. At death or the conclusion of the study, animals were euthanized with intravenous sodium pentobarbital 100 mg/kg.

Whole blood cyanide levels, which includes cyanide bound to cobinamide and hydroxocobalamin, were measured spectrophotometrically at a referral laboratory (Michigan State University, Diagnostic Center for Population and Animal Health, Lansing, MI).[11] This method generates hydrogen cyanide gas, converts it to a cyanogen chloride, and measures a barbituric acid complex.[10,11]

Methods of Measurement and Outcome Measures

The primary outcome was time to return of spontaneous breathing after 1 minute of cyanide-induced apnea. This outcome was defined before the study and based on our previous research.[3,7,12] We also assessed survival and compared cardiac output, pulse rate, mixed venous oxygenation, pH, lactate, base excess, serum bicarbonate, cyanide concentrations, and inflammatory markers. Vital signs and hemodynamic measurements were recorded at 1-minute intervals and analyzed at 5-minute intervals. Blood sampling was obtained at baseline, 5 minutes after start of the cyanide infusion, at the onset of apnea, after 1 minute of apnea, and at 10, 20, 30, 40, 50, and 60 minutes after treatment.

Primary Data Analysis

The average time (in minutes and seconds) to return of spontaneous breathing after the antidote/saline solution was administered was compared among the 3 groups with the Kruskal-Wallis test. This nonparametric statistical analysis was used because the data were not normally distributed (Shapiro-Wilk test). Additional analysis was performed among the 3 groups to assess the differences in time to death, using Kaplan-Meier estimation methods of the survival distribution and log-rank testing to compare survival among the groups.

Secondary outcome variables (cardiac output, pulse rate, systemic vascular resistance, respiratory rate, mean arterial blood pressure, mixed venous oxygenation, and inflammatory markers) were modeled with repeated-measures ANOVA, with adjustment for treatment, time, and the interaction of treatment by time with an autoregressive covariance structure assumed. Because only 2 of the control animals survived after 30 minutes, the control group was not included in secondary outcome variable analysis so that those data would not unduly influence the results of the comparison between the 2 antidote intervention groups. Post hoc analysis was performed on all variables that showed a significant treatment-by-time interaction, for which treatment contrasts were measured at each posttreatment point with a Bonferroni adjustment for multiple testing applied. Values for arterial blood pH, lactate, cyanide, bicarbonate, base excess, and potassium concentrations were compared among groups with repeated-measures ANOVA for times zero to 60 minutes.

All statistical testing was 2 sided, with a significant level of $\alpha=.05$, and was completed with SAS (version 9.3; SAS Institute, Inc., Cary, NC). All graphic presentations were made with R version 2.15.1. Sample size calculations were based on our previous animal experiments of acute cyanide toxicity. A sample size of 11 animals per group was determined to be sufficient according to obtaining a power of 80%, an $\alpha=.05$, and an SD of 0.17 (based on previous experiments) in mean time to detect a 20% difference in time to spontaneous breathing. Sample size calculation was performed with PASS 12 (version 12; NCSS, LLC, Kaysville, UT; http://www.ncss.com).

RESULTS

Characteristics of Study Subjects

At baseline and at apnea, the groups had similar vital signs and biochemical variables (Tables 1 and 2). At time zero, predefined as apnea of 1 minute's duration, there were no significant differences among groups (Table 3). Reduction in mean arterial blood pressure from baseline was also similar among groups (29%, 38%, and 36% decrease; $P=.35$).

Main Results

The time to return of spontaneous breathing between the 2 antidote-treated groups was similar: hydroxocobalamin group (1 minute 48 seconds [SD 29 seconds]), and cobinamide group (1 minute 49 seconds [SD 31 seconds]). This was significantly different from the control group (5 of 11 animals had return of spontaneous breathing and 6 of 11 remained apneic; 4 minutes 5 seconds [SD 40 seconds]; $P=.005$). One animal in the hydroxocobalamin group, 1 animal in the cobinamide group, and 9 animals in the control group died before completion of the experiment, ie, between receiving the antidote or saline solution at 1 minute after onset of apnea and 60 minutes later (Figure 1). Consequently, the 3 groups showed a difference in the Kaplan-Meier survival estimation (90% survival in hydroxocobalamin and cobinamide animals, 10% in control) and time to death compared with controls ($P<.001$).

Table 1. Baseline characteristics of animals in each group before receiving cyanide infusion.*

Characteristics at Baseline	Hydroxocobalamin (65 mg/kg IV) (N=10)	Cobinamide (12.5 mg/kg IV) (N=10)	Control (Saline Solution) (N=11)
Weight, kg	49.9 (3.9)	52.7 (3.09)	51 (2.86)
Pulse rate, beats/min	76.7 (10.69)	84.9 (14.72)	85.55 (23.56)
Systolic blood pressure, mm Hg	122.9 (12.42)	120.7 (16.46)	119.36 (16.75)
MAP, mm Hg	99.2 (12.35)	97.9 (13.86)	96.09 (17)
Cardiac output, L/min	4.97 (0.98)	6.03 (1.13)	5.13 (1.28)
Systemic vascular resistance, dynes-s/cm^5	1,564.2 (464.9)	1,244.9 (221.32)	1,418.9 (303.27)
pH, mEq/L	7.46 (0.02)	7.47 (0.03)	7.47 (0.02)
Bicarbonate, mEq/L	27.81 (1.26)	27.56 (2)	28.43 (2.24)
Lactate, mmol/L	1.06 (0.38)	1.1 (0.46)	0.89 (0.31)

IV, Intravenous, *MAP*, mean arterial pressure.
*Data are presented as mean (SD).

Outcome variables for the control group are reported until more than half of the animals died, which occurred at 30 minutes after time zero. Of the antidote-treated animals that survived, respiratory rate, mean arterial pressure, pulse rate, cardiac output, and central mixed venous oxygenation all trended toward baseline after treatment (Figure 2A through E and Figure E2 available at www.annemergmed.com)). There were no significant differences in respiratory rate, cardiac output, or mixed venous oxygenation between treatment groups from time zero to 60 minutes. Mean arterial pressure was significantly different between the 2 antidote-treated groups (*P*<.05) such that pigs in the hydroxocobalamin-treated group demonstrated an increased mean arterial pressure at 5 minutes through 50 minutes postapnea. Moreover, pulse rate was significantly faster at times 5 to 15 minutes in the hydroxocobalamin-treated animals compared with cobinamide-treated animals. However, post hoc analysis at the individual times revealed no statistical difference by the end of the experiment (88 [SD 15.2] 15.2 mm Hg hydroxocobalamin and 71 [SD 10] 10 mm Hg cobinamide). The systolic blood pressure was also different between groups (*P*<.05) but similar at the end of the experiment (112 mm Hg

Table 2. Characteristics for each group at apnea of the cyanide-poisoned animals.*

Characteristics at Apnea	Hydroxocobalamin (65 mg/kg IV) (N=10)	Cobinamide (12.5 mg/kg IV) (N=10)	Control (Saline Solution) (N=11)
Cyanide dose, mg/kg	1.91 (0.7)	1.85 (0.53)	1.67 (0.53)
Time to apnea, min:s	11:12 (04:04)	10:54 (03:07)	09:49 (03:05)

*Data are presented as mean (SD).

Table 3. Characteristics for each group at time zero (1 minute of apnea) of the cyanide-poisoned animals.*

| Characteristics at 1 Minute of Apnea	Hydroxocobalamin (65 mg/kg IV) (N=10)	Cobinamide (12.5 mg/kg IV) (N=10)	Control (Saline Solution) (N=11)
MAP at apnea, mm Hg	70 (17)	61 (20)	58 (27)
Lactate, mmol/L	3.4 (1.3)	3.7 (0.9)	3.2 (1.1)
Cyanide level, µg/mL	1.83 (0.51)	1.68 (0.36)	1.76 (0.59)
pH	7.38 (0.05)	7.36 (0.05)	7.38 (0.07)

*Data are presented as mean (SD).

SD [14.1] 14.2 mm Hg IV hydroxocobalamin, 91 [SD 15.1] mm Hg IV cobinamide).

No important difference was detected between treated groups in regard to lactate, bicarbonate, pH, or cyanide concentration levels from time zero through the end of the study (Figure 3A through D and Figure E3 available at www.annemergmed.com)). Lactate (1.2 versus 1.5 mmol/L), pH (7.44 versus 7.44), and bicarbonate (28 versus 28 mEq/L) at 60 minutes were similar in the treated groups. Immediately after treatment, cyanide was not detected in the blood of 10 of 10 hydroxocobalamin-treated animals and 7 of 10 cobinamide-treated animals. Cyanide was not detectable in any treated animal at the end of the study. The likely reason that blood cyanide was detected longer in the cobinamide-treated animals than the hydroxocobalamin-treated animals is that cobinamide binds more tightly to plasma proteins than hydroxocobalamin; thus, cobinamide was likely at a higher blood concentration than hydroxocobalamin, yielding higher cyanide concentrations.

LIMITATIONS

This study has several limitations, the principal one being that an animal model does not precisely reproduce human toxicity.
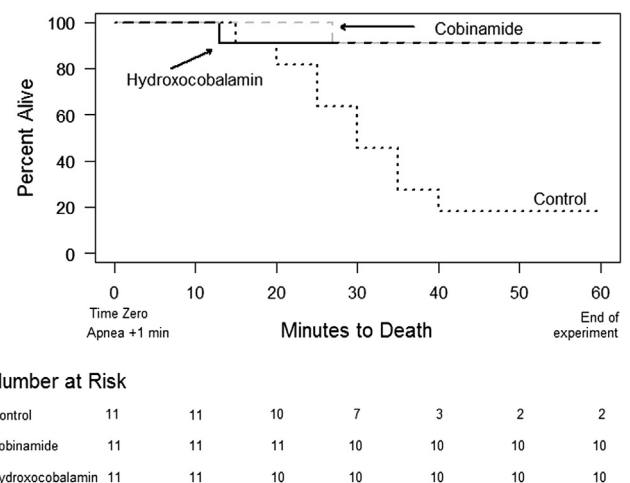
Figure 1. Survival analysis using a Kaplan-Meier curse plot estimate comparing all the groups of cyanide-poisoned animals.
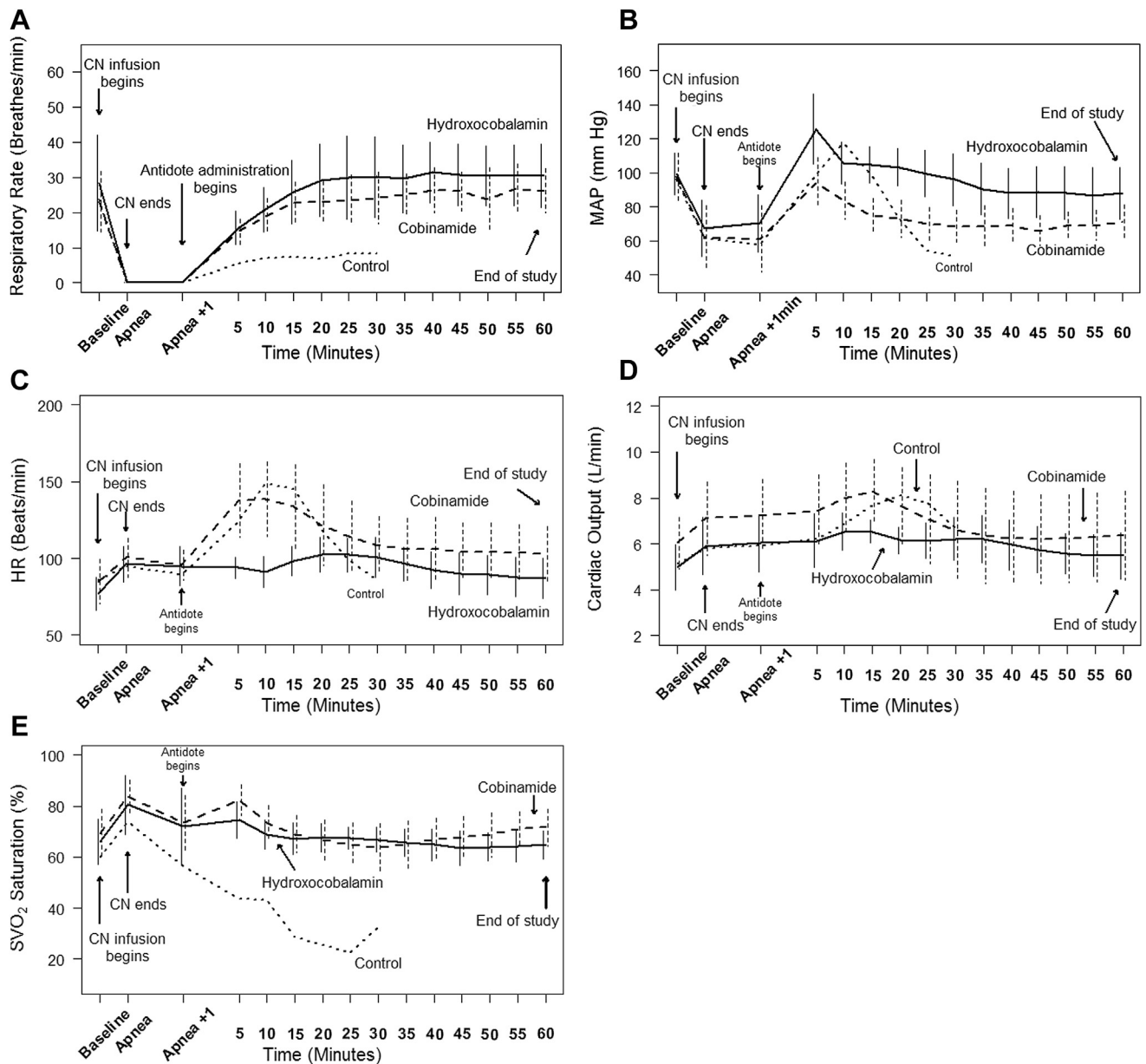
Figure 2. Hemodynamic variables and vital signs (respiratory rate, mean arterial pressure, pulse rate, cardiac output, and mixed venous oxygenation [SVO$_2$] saturation) in cyanide-poisoned animals over time for the 3 groups. Values for the control arms were plotted until greater than 50% of the animals died (30 minutes). *MAP*, Mean arterial pressure; *HR*, pulse rate; *CN*, cyanide.

However, it clearly is not possible to administer cyanide to humans, and animal models must be used. We have previously noted that pigs are an excellent choice for modeling cyanide exposure, given the similarities of their cardiovascular systems to that of humans.[3,13,14]

Another shortcoming is that we used intravenous cyanide as a substitute for inhalational exposure. Both routes have rapid onset, but the intravenous route provides a controlled method to induce toxicity compared with relatively uncontrolled cyanide absorption in an inhalational model. In addition, an inhalational route of cyanide exposure for a large animal puts the research staff

at a greater risk than the intravenous route because of the potential for undetected leaks in the ventilation system.[10,12,15]

A third potential concern is that we used potassium cyanide, rather than sodium cyanide. However, the potassium dose received was small, about 0.67 mEq during 10 minutes.

A fourth limitation is that we observed the animals for only 60 minutes after treatment. A longer observation period may have shown a difference between the 2 antidote-treated groups.

Finally, our study was not blinded; however, we reported objective criteria (death, breathing-based capnography, blood
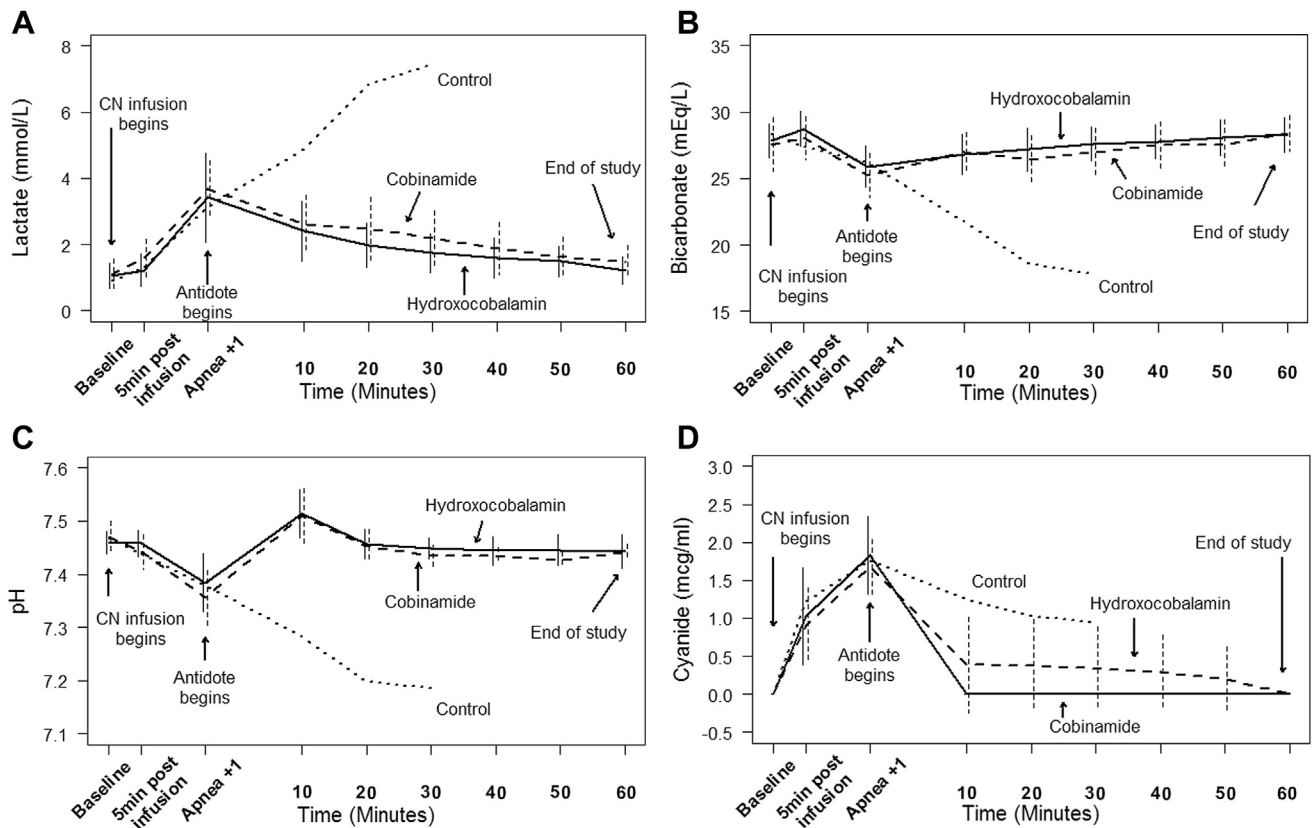
Figure 3. Serum markers (lactate, bicarbonate, pH, and cyanide concentrations) of cyanide-poisoned animals over time for the 3 groups. Values for the control arms were plotted until greater than 50% of the animals died (30 minutes).

pressure, and cyanide levels) to limit the subjectivity of interpretation of the results.

DISCUSSION

We expected cobinamide to provide a significantly faster and more complete rescue for cyanide-exposed animals compared with either hydroxocobalamin or saline solution. Previous investigations in our laboratory comparing the 2 antidotes in mice and rabbits suggested that cobinamide is 3 to 10 times more potent than hydroxocobalamin as a cyanide antidote, depending on the cyanide exposure model.[7,8] To our knowledge, this is the first investigation comparing the antidotes in a pig model of cyanide poisoning. We found no difference between cobinamide, an agent being developed as a cyanide antidote, and hydroxocobalamin, an established cyanide antidote, in terms of the primary outcome measure of time to return of spontaneous breathing after cyanide-induced apnea. Furthermore, we found both groups similar in terms of mortality, lacticemia, acidosis, and clearance of cyanide. Although we found differences in mean arterial pressure and pulse rate between the 2 antidote-treated groups, we find it difficult to speculate about these minor cardiovascular differences because this is a small controlled animal study. Moreover, these differences had abated by the conclusion of the study.

Although no difference was noted between cobinamide and hydroxocobalamin in terms of the main outcome variable, the dose of cobinamide we used to rescue the animals was one fifth that of hydroxocobalamin, on a milligram-per-kilogram basis. This apparent increased potency may relate to the fact that each cobinamide molecule can bind, and therefore neutralize, 2 cyanide molecules compared with hydroxocobalamin, which can bind only 1 cyanide molecule.[6,8,16] This difference in binding capacity explains only a 2-fold increase in potency, suggesting other differences between cobinamide and hydroxocobalamin as cyanide antidotes. Two other explanations seem likely. First, cobinamide has a much higher affinity for cyanide than hydroxocobalamin ($K_{aoverall}$ 10^{22} M^{-2} for cobinamide and K_a 10^{12} M^{-1} for hydroxocobalamin).[17] This could allow cobinamide to more effectively remove cyanide from cytochrome c oxidase than hydroxocobalamin; we have previously shown that cobinamide is more potent than hydroxocobalamin in reversing cyanide inhibition of cellular respiration.[6] Cytochrome c oxidase is one of the primary molecular targets of cyanide and is part of complex IV of the mitochondrial electron transport system. Second, we have preliminary evidence that cobinamide is transported into cells more rapidly and completely than hydroxocobalamin. Together, these 3 differences between cobinamide and hydroxocobalamin of increased cyanide binding capacity, increased cyanide

binding affinity, and increased cellular transport could explain the apparent increased potency of cobinamide as a cyanide antidote.

There are 2 major differences between our study and previous work on animal models of cyanide poisoning. First, we used 50-kg Yorkshire pigs instead of rodents, rabbits, or dogs, which have been more commonly used in studies of cyanide toxicity.[7,12,15,18] Second, ours was a strictly nonventilated model.

We chose pigs because they are close in size to humans, thereby minimizing scaling issues, and because their cardiovascular system is similar to that of humans.[3,13,14] Drug doses are generally converted from one species to another with body surface area, but this may not be appropriate for all drugs, and less scaling will usually lead to a more accurate estimate of the proper human dose. The heart and brain are generally considered the 2 primary targets of cyanide, and thus, using an animal model that has a cardiovascular system close to that of humans allows one to apply the results to humans more confidently. In addition, we have reported swine models of cyanide-induced cardiac arrest and of shock.[3,9,10]

In the out-of-hospital setting of a major cyanide exposure such as a massive fire or a terrorist attack, it is extremely unlikely that sufficient time or resources will be available to intubate and ventilate the large number of cyanide-exposed victims.[8] Thus, we chose not to ventilate our animals as a means to more accurately simulate such a scenario. This considerably narrows the window of time available to rescue an animal from cyanide exposure, and we elected to treat the pigs after 1 minute of apnea. Although this could be considered a relatively short period of apnea, it is not realistic to think that humans can be rescued fully after several minutes of apnea. We believe that the advantages of simulating a mass casualty scenario of no artificial ventilation outweigh the disadvantage of treatment after 1 minute of apnea.

Now that we have shown that intravenous cobinamide is comparable to intravenous hydroxocobalamin in a swine model of acute cyanide toxicity, the next step is to evaluate cobinamide delivered by nonintravenous routes. To that end, we currently have multiple ongoing swine studies evaluating the efficacy of intraosseous and intramuscular administration of cobinamide in a similar model.

In conclusion, no difference was noted between intravenous cobinamide, at one fifth the dose, and intravenous hydroxocobalamin for return of spontaneous breathing after acute cyanide poisoning in a nonventilated swine model.

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MEMS-Based Silicon Ultrasonic Twin-Nozzle Nebulizer for Inhalation Drug Delivery*

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Abstract— **A versatile silicon-based ultrasonic nebulizer that utilizes a twin-nozzle of multiple Fourier horns at 1—2 MHz drive frequencies has been realized to perform simultaneous aerosolization of cobinamide and magnesium thiosulfate drug solutions. The drive frequency of the individual nozzle for a desirable aerosol diameter was individually designed. Using the 2.0 MHz 4-Fourier horn twin-nozzle aerosols of the two drug solutions with mass median diameter (MMD) of 3.0±0.1µm and geometrical standard deviation (GSD) of 1.18±0.02 and total flow rate up to 400µL/min were produced.**

Keywords— *MEMS, Multiple Fourier-horn Nozzle, Twin-Nozzle Ultrasonic Nebulizer, Inhalation Drug Delivery, Medicinal Aerosol Mixing*

I. INTRODUCTION

The innovation and potential applications of megahertz (MHz) MEMS-based multiple-Fourier horn ultrasonic nozzles that utilize temporal instability of Faraday waves for formation and ejection of monodisperse micro droplets were highlighted in a new journal most recently [1]. In fact, the resulting ultrasonic nebulizer module using a single nozzle was used successfully to aerosolize a number of common pulmonary drugs [1, 2]. Controllability of particle (aerosol) size range (2 to 6µm) and much narrower size distribution achievable by the new device will improve targeting of treatment within the respiratory tract and improve delivery efficiency. For example, a recent in-vitro experiment with Technetium (T_c)-tagged saline solution using the new nebulizer module has demonstrated higher delivery efficiency than the existing commercial nebulizers [3]. Therefore, it may constitute a desirable device for inhalation delivery of expensive medicines such as gamma interferon [4]. Short treatment time is a critical requirement in situations such as massive cyanide poisoning [5]. Clearly, the treatment time can be shortened by increased aerosol output rate of an array of such nozzles. Furthermore, nozzle arrays with individual nozzles operating at identical or different drive frequency will provide the unique capability for formation and subsequent mixing of medicinal aerosols of the same or different medicines at identical or different aerosol sizes. Note that such strategy is essential in order to avoid instability of mixed drug solutions prior to aerosolization. Here we report the realization of a twin-nozzle array and its unique capability via simultaneous nebulization of two drug solutions in-vitro towards ultimate inhalation drug delivery to human lung.
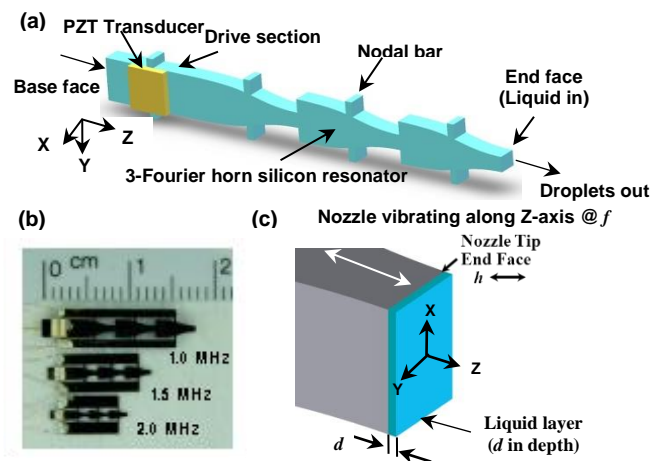
Fig. 1 (a) 3-D architecture of the MHz multiple-Fourier horn ultrasonic nozzle; (b) Photograph of the 1.0, 1.5, and 2.0 MHz nozzles; (c) Geometry of nozzle end face vibrating at nozzle drive frequency *f* with displacement *h* and liquid layer *d* in depth.

II. TWIN-NOZZLE ULTRASONIC NEBULIZER

A. Architecture of Element Nozzle and Working Principle

Each basic (element) nozzle consists of a drive section and a resonator section (Fig. 1a). A lead zirconate titanate (PZT) transducer is bonded on the drive section to excite mechanical vibrations along the nozzle axis. The resonator section is made of multiple (3 in the example) Fourier horns in cascade [1]. The nozzle is designed to vibrate in a single longitudinal mode at the resonance frequency of the multiple Fourier horns. The resonance effect greatly enhances the vibration displacement (*h*) of the nozzle end face (by a factor of approximately 8 for three Fourier horns) and, hence, readily facilitates excitation and subsequent temporal instability of Faraday waves on the liquid layer resting on the nozzle end face. Droplets are formed and ejected from the nozzle end face when its longitudinal

vibration displacement exceeds the onset threshold [6]. The liquid to be atomized is externally transported to the nozzle end face using a fused silica tube. Importantly, the single vibration mode at the single MHz resonance frequency ensures single-mode capillary wave atomization mechanism, namely, temporal instability of Faraday waves, and production of micron-size monodisperse droplets at very low electrical drive power [1]. The diameter (D_p) of the resulting droplets is equal to four tenth of the wavelength (λ) of the Faraday waves excited as follows [6]:

$$D_p = 2(2/\pi^2)^{1/3}(\sigma/\rho)^{1/3} f^{-2/3} = 0.40\lambda, \qquad (1)$$

where σ, ρ, and f designate surface tension, liquid density, and the nozzle drive frequency (resonance frequency), respectively.
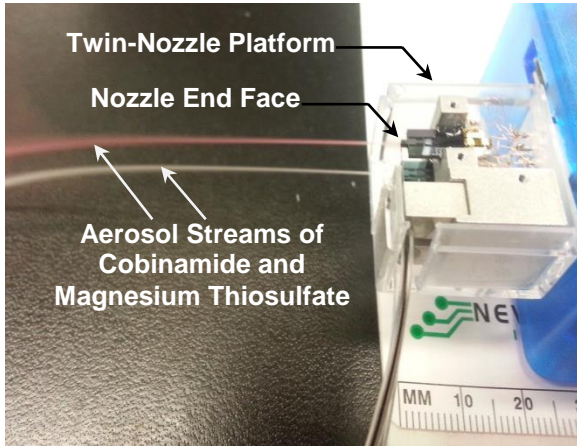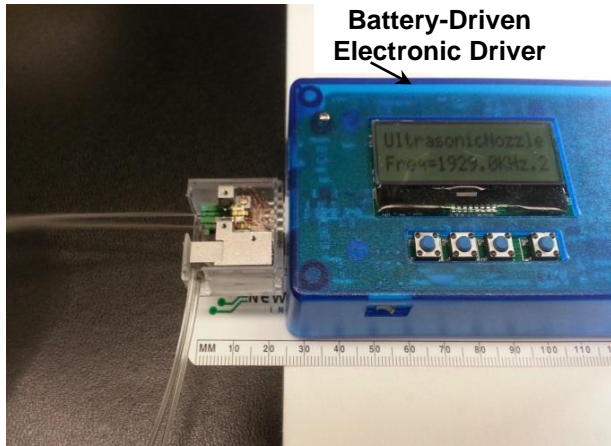

Fig. 2 Twin-nozzle platform

Fig. 3 Pocket-size MEMS-based ultrasonic twin-nozzle nebulizer

B. Platform of Twin-Nozzle and Pocket-Size Nebulizer

Fig. 2 shows the platform for installation of the twin-nozzle array with identical or separate design specifications for the aerosol size (or drive frequency). The twin nozzles were driven by a pair of independent electronic drivers with controllable frequency and power together with separate fused silica tubes for transport of drug solutions to the nozzle end faces. Fig. 3 shows the resulting battery-run pocket-size ultrasonic nebulizer.

III. SIMULTANEOUS NEBULIZATION OF IDENTICAL OR DIFFERENT MEDICINES

Separate aerosolization of 100mM cobinamide solution [7] and 1.0 M magnesium thiosulfate solution as well as simultaneous aerosolization of the two drug solutions at varying flow rates were carried out using the nebulizer shown in Figs. 2 and 3 with 2 MHz 4-Fourier horn twin-nozzle and established equipment and characterization procedures [8]. Fig. 2 shows the two streams of aerosols produced simultaneously at respective flow rates of 200µL/min and 250µL/min.
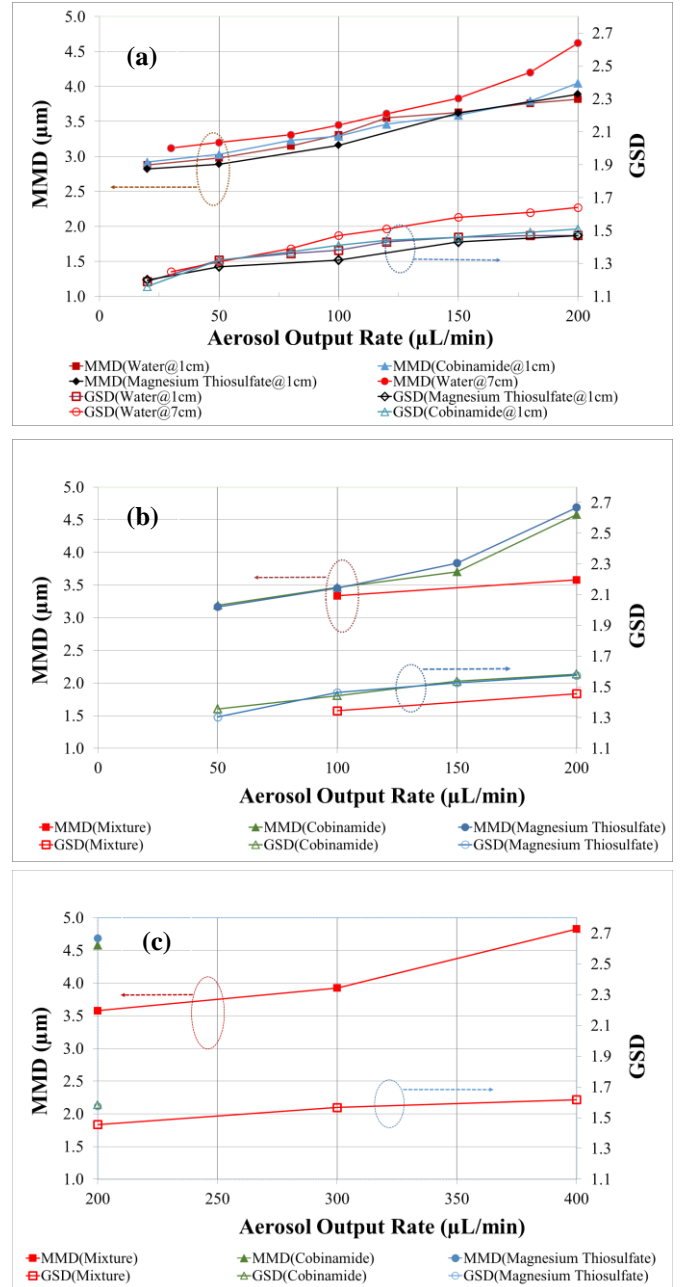

Fig. 4 Measured aerosol sizes in mass median diameter (MMD)/geometrical standard deviation (GSD) versus output rate for 100mM cobinamide solution and 1 M magnesium thiosulfate solution using the 2 MHz 4-Fourier horn ultrasonic nebulizers with (a) single-nozzle, and (b) and (c) twin-nozzle. Note that all data in (b) and (c) were obtained at a distance of 7 cm from the nozzle end face.

The sizes and size distributions of the aerosols produced were measured using Malvern/Spraytec system (Model #STP 5311) which is a well-established non-invasive particle sizing instrument based on laser light diffraction. The streams of aerosol traveled from the nozzle end faces (as depicted in Figs. 2 and 3) and passed the laser beam of the instrument. Fig. 4(a) shows that the sizes of aerosols produced using the 2 MHz single-nozzle nebulizer and measured at 1 cm from the nozzle end face are within the experimental errors in sizes for all three liquids including water (reference liquid), cobinamide solution, and magnesium thiosulfate solution. Specifically, the measured mass median diameters (MMDs) of the aerosols of water and the two aqueous drug solutions are in good agreement with the predicted value of 3.1μm for water based on Eq. (1). The MMDs are seen in Fig. 4(a) to increase from 2.8±0.1 to 3.8±0.2μm as the aerosol output rate (liquid flow rate) increases from 20 to 200μL/min. The corresponding geometrical standard deviation (GSD) increases from 1.18±0.02 to 1.49±0.02. Fig. 4(a) also shows that the sizes of the water (reference liquid) aerosols measured at a distance of 7 cm are slightly larger than those measured at a distance of 1 cm. The increase in aerosol sizes with increased output rate may be caused by aerosol coalescence in the dense sprays.

A comparison of Fig. 4(b) with Fig. 4(a) shows that like the water aerosols, the aqueous medicinal aerosols measured downstream at 7 cm from the nozzle end faces of the 2 MHz twin-nozzle nebulizer are slightly larger than those measured at 1 cm from where they were produced (liquid layer on the nozzle end face). The two streams of aerosols ensuing from the two nozzle end faces overlapped (mixed) at 7cm downstream. Note that the aerosol MMD and GSD were measured at both 1 and 7 cm from the nozzle end faces.

Furthermore, Figs. 4(b) and 4(c) show that when the two streams of aerosols overlapped at 7 cm from the nozzle end face, the total aerosol output rate was doubled to about 400μL/min, and the MMD and GSD of the mixed aerosols were slightly larger than those of the individual aerosol streams at half the total output rate.

IV. CONCLUDING REMARKS E

A pocket-size ultrasonic nebulizer with twin-nozzle of silicon multiple-Fourier horn has been realized to demonstrate the capability of doubling the aerosol output of same drug solution and simultaneous aerosolization of different drug solutions. Specifically, for 100mM cobinamide and 1 M magnesium thiosulfate drug solutions, simultaneous and continuous aerosolization at respective flow rates of 200μL/min and 250μL/min for 7 min. delivered 430mg thiosulfate and 152mg cobinamide that would be nearly sufficient antidote dosages for effective detoxification of cyanide poisoning.

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